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ORIGINAL ARTICLE

Five New Alkaloids from *Cephalotaxus lanceolata* and *C. fortunei var. alpina*

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Abstract Five new alkaloids (**1–5**) were isolated from the leaves and twigs of *Cephalotaxus lanceolata* and *C. fortunei var. alpina* along with 24 known alkaloids. The new structures were elucidated based on spectroscopic data including 1D and 2D NMR, FTIR, UV and MS. These new alkaloids showed no cytotoxicity to HeLa, SGC-7901 gastric cancer, and A-549 lung cancer cell lines.

Keywords *Cephalotaxus* · Alkaloids · Cytotoxicity

1 Introduction

Various constituents of *Cephalotaxus* genus have been reported, including alkaloids [1–6], tropones [7–10], lignans [10, 11], diterpenes [9], flavonoids [6, 10]. Previous investigations led to approximate 100 *Cephalotaxus* alkaloids, which were mainly classified into two structural types, i.e., homoerythrina and cephalotaxine-type, and the latter demonstrated remarkable antitumor activities [12]. For example, homoharringtonine among cephalotaxine alkaloids was successfully used to treat acute leukemia. As for homoharringtonine, the side chains played an important role in the anticancer activity of these compounds which possessed H-3 α-configuration. So far only reported cephalezomines G possessed H-3 β-configuration. Both homoerythrina and cephalotaxine had same biogenetic origin. However, most of homoerythrinas almost with H-3 α-configuration reminded us that there were more cephalotaxines with same configuration. As a part of our continuous research for *Cephalotaxus* alkaloids, five new alkaloids, together with 24 known ones (Fig. 1) were isolated from leaves and twigs of *C. lanceolata* and *C. fortunei var. alpina*. The known alkaloids were identified as drupacine (**6**) [2], cephalotaxinone (**7**) [13], acetycephalotaxine (**8**) [14], cephalezomine J (**9**) [5], desmethylcephalotaxine (**10**) [15], isocephalotaxinone (**11**) [16], 1l-hydroxycephalotaxin (**12**) [2], cephalotaxine (**13**) [17], lucidinine (**14**) [18], comosidine (**15**) [18], schelhammeridine (**16**) [19], 3-epischelhammeridine (**17**) [20], comosine (**18**) [21], 3-epicomosine (**19**) [20], 3-epischelhammericine (**20**) [20], fortunine (**21**) [22], taxodine (**22**) [23], *O*-methylschlammericine (**23**) [13], cephalezomine M (**24**) [5], homoisoharringtonine (**25**) [24], homoharingtonine (**26**) [25],

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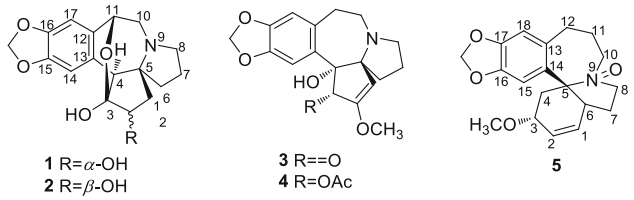

Fig. 1 Structures of alkaloids from *C. lanceolata* and *C. fortunei var. alpine*

isoharringtonine (**27**) [25, 26], epidesoxyharringtonine (**28**) [27], desoxyharringtonine (**29**) [28] by comparison with literatures.

2 Results and Discussion

Newly isolates (**1**–**5**) probably belong to alkaloids as they exhibited a positive reaction with Dragendorff's reagent. Alkaloid **1** was isolated as white powder. Its UV absorption bands at 203 and 291 nm and IR absorption bands at 3520, 3406, 1631, 1500, 1482, 1342 cm^{-1} were consistent with those of *Cephalotaxus* alkaloids [2]. Analysis of the ^{1}H and ^{13}C NMR data of **1** (Tables 1, 2) revealed several typical functionalities similar to those of the known alkaloid drupacine (**6**) [2], including a tetrasubstituted benzene ring with two *para* H-atoms (δ_H 6.76, δ_C 110.1; δ_H 6.72, δ_C 106.0; δ_C 128.6, 132.4, 146.8, 147.4), a $-OCH_2O-$ moiety (δ_H 5.97; δ_C 101.5), a ketal carbon (δ_C 106.7), two *O*-bearing CH groups (δ_H 3.86, δ_C 76.7; δ_H 4.81, δ_C 76.1), and two –OH groups (δ_H 3.53 and 4.68). The molecular formula of **1** was established as $C_{17}H_{19}NO_5$ with nine degrees of unsaturation by HRESIMS ($[M+H]^+$ at *m/z* 318.1336), absence of a methyl than that of **6**. The HMBC correlations (Fig. 2) of the methine signal (δ_H 4.81) with C-12 (δ_C 132.4), C-13 (δ_C 128.6), and C-17 (δ_C 106.0) allowed its position as C-11. Likewise, the other signal δ_H 3.38 was assigned to CH-4 based on its HMBC correlations with δ_C 110.1 (C-14), C-12 and δ_C 39.2 (C-6). The obvious HMBC correlation between methylene protons (δ_H 1.37 and 2.23) with C-6 and C-3 attributed it to C-1. The proton signal δ_H 3.86 was assigned to H-2 based on its correlation with δ_H 2.23 in the ^{1}H–^{1}H COSY (Fig. 2) spectrum. The ketal carbon (δ_C 106.7) was located at C-3 by its HMBC correlations from H-1, 2 and 4. The HMBC crosspeak of H-11/C-3 showed an oxygen bridge between C-11/C-1 in **1** consistent with its degrees of unsaturation. H-2 was established as β-orientation on the basis of the coupling constant (d, $J = 6.4$ Hz) of H-2. Consequently, the structure of **1** was confirmed as shown in Fig. 1, and named cephalotine A.

Alkaloid **2** had the same molecular formula (HRESIMS *m/z* 318.1335 $[M+H]^+$) and very similar UV and IR spectra as **1**. Comparison of the ^{13}C NMR data of **2** and **1** (Table 2) suggested that both compounds shared the same planar structure. In the ^{1}H NMR spectrum (Table 1), obvious difference between both alkaloids was that a proton signal δ_H 3.86 (d, $J = 6.4$ Hz, H-2) in **1** was replaced by δ_H 4.05 (t, $J = 8.9$ Hz) in **2**. This indicated α-configuration of H-2 in **2**, and confirmed by a ROESY correlation from H-2 to H-4. Thus, **2** was established as 3-epi-cephalotine A and named cephalotine B.

Alkaloid **3** displayed similar ^{1}H and ^{13}C NMR data (Tables 1, 2) to the known alkaloid cephalotaxinone (**7**) [13] except that a quaternary carbon (δ_C 81.9) in **3** substituted a methine in **7**. In addition, the HMBC correlations of both H-1 and H-14 with δ_C 81.9 located the quaternary carbon to C-4. The molecular formula $C_{18}H_{19}NO_5$ of **3** from HRESIMS *m/z* at 330.1337 $[M+H]^+$, 16 mass units higher than that of **7**, further indicated that **3** was an 4-hydroxy cephalotaxinone. Alkaloid **4** showed the similar ^{13}C NMR data to the known alkaloid acetycephalotaxine (**8**) [14], except that a methine signal of **8** was substituted by a quaternary carbon δ_C 86.1 (s) in **4**. Like in **3**, the additional hydroxyl of **4** was also located at C-4 by its molecular formula $C_{20}H_{23}NO_6$ by HRESIMS at *m/z* 374.1604 $[M+H]^+$), 16 mass units higher than that of **8**. Further, this was supported by the HMBCs of δ_H 5.21 (H-1) and δ_H 7.15 (H-14) with δ_C 86.1 (C-4). The hydroxyl of **3** and **4** adopted α-orientation by the molecular model. The configuration of H-3 in both alkaloids was α-oriented by ROESY correlation between H-3 and H-11. Therefore, **3** and **4** were named cephalotines C and D, respectively.

Six methylenes, 3 methines, a methyoxyl and 5 quaternary carbons in the ^{13}C NMR spectrum of alkaloid **5** revealed that **5** belongs to homoerythrina-type alkaloids rather than cephalotaxine-type alkaloids [2]. The ^{13}C NMR and DEPT data of alkaloid **5** were similar to those of comosine (**18**) [21] with exception for three downfielded signals [87.0 (s), 67.6 (t), 63.3 (t)], suggesting a *N*-oxide moiety. Additionally, its molecular formula $C_{20}H_{23}NO_4$ by HRESIMS (*m/z* 330.1717 $[M+H]^+$) could support this presumption. The H-3 was allowed at β-configuration through ROESY correlations of H-3 with H-10 and H-12. Thus **5** was named as cephalotine E.

None of these compounds showed any significant activity against HeLa, SGC-7901 gastric cancer, and A-549 lung cancer cell lines ($IC_{50} > 20$ μM).

3 Experimental Section

3.1 General Experimental Procedures

Optical rotations were carried out using a Horiba SEPA-300 polarimeter and *J*ASCO DIP-370 digital polarimeter.

Table 1 ^{1}H NMR spectroscopic data of **1–5** (δ in ppm and J in Hz)

Position	δ_H(**1**)[a]	δ_H(**2**)[a]	δ_H(**3**)[b]	δ_H(**4**)[a]	δ_H(**5**)[a]
1	1.37 d (15.0)	1.70 dd (14.4, 8.4)	6.69 s	5.21 s	6.12 m
	2.23 dd (15.0, 6.4)	1.82 dd (14.4, 9.6)			
2	3.86 d (6.4)	4.05 t (8.9)			5.74 d (10.2)
3			–	5.47 s	2.88 overlap
4	3.38 s	3.10 s	–	–	1.66 overlap
					2.92 overlap
6	1.67 overlap	1.58 overlap	1.79 m	1.65 overlap	3.93 overlap
	1.69 overlap	1.68 m	2.20 dd (11.4, 4.2)	2.30 m	
7	1.55 overlap	1.56 overlap	1.64 m	1.49 overlap	1.70 overlap
	1.57 overlap	1.58 overlap	1.82 m	1.63 overlap	2.64 m
8	2.28 m	2.25 m	2.80 m	2.67 overlap	3.54 td (2.4, 13.2)
	2.60 td (8.8, 3.6)	2.59 m	2.95 overlap	2.95 m	4.08 m
10	2.55 d (12.3)	2.65 d (12.2)	2.61 dd (11.4, 7.8)	2.65 overlap	3.43 d (13.0)
	2.71 dd (12.3, 4.0)	2.71 dd (12.2, 4.0)	2.75 m	2.70 m	3.92 overlap
11	4.81 d (3.1)	4.87 d (3.8)	2.37 m	2.36 dd (14.4, 6.8)	1.68 overlap
			2.49 dd (15.0, 7.8)	3.12 m	2.57 m
12					2.77 dd (5.8, 15.7)
					3.31 td (2.3, 11.2)
14	6.76 s	6.73 s	7.31 s	7.15 s	
15					6.62 s
17	6.72 s	6.74 s	6.63 s	6.67 s	
18					6.67 s
OCH_2O	5.97 s	5.93 d (1.1)	5.96 s	5.93 s	5.93 s
	5.93 s	5.97 d (1.1)	5.97 s	5.96 s	5.94 s
2-OH	3.53 br*	3.45 br*	–		
3-OH	4.68 br*	4.65 br*	–		
4-OH	–	–	5.11		
2-OCH_3	–	–	3.81 s	3.66 s	
3-OCH_3					3.22 s
CH_3CO	–	–	–	2.51 s	

Alkaloids **1**, **2**, **4** and **5** recorded in acetone-d_6; **3** in DMSO-d_6

* Assignments may be interchanged

[a] Recorded at 400 MHz

[b] Recorded at 600 MHz

UV spectra were recorded on Shimadzu 2401Aspectrophotometer. IR Spectra were obtained on Brucker Tensor 27 infrared spectrophotometer with KBr pellets. ^{1}H, ^{13}C and 2D NMR spectral data were measured on a Bruker Avance III-600, DRX-500, and AM-400 MHz spectrometers with $SiMe_4$ as an internal standard. HRESIMS data were recorded on an Agilent G6230 TOF MS. Column chromatography (CC) was performed with silica gel (200–300 mesh, Qing-dao Haiyang Chemical Co., Ltd., Qingdao, China). RP-18 silica gel (20–45 μm, Fuji Silysia Chemical Ltd., Japan). Fractions were monitored by TLC on silica gel plates (GF254, Qingdao Haiyang Chemical Co., Ltd.) and spots visualized with Dragendorff's reagent spray. MPLC was employed using a Buchi pump system coupled with RP-18 silica gel packed glass columns(15 × 230 and 26 × 460 mm, respectively). HPLC system was carried out on a Waters HPLC system (Waters 1525E pumps, Waters 2996 photodiode array detector, Waters fraction collector II) using a analytical semi-preparative or preparative Sunfire C_{18} column (4.6 × 150, 10 × 150, and 19 × 250 mm, respectively).

3.2 Plant Materials

Leaves and stems of *C. lanceolata* and *C. fortunei var. alpina* were collected from Yunnan Province, P. R. China

Table 2 ^{13}C NMR spectroscopic data of alkaloids **1–5** (δ in ppm)

Position	δ_C(**1**)[a]	δ_C(**2**)[a]	δ_C(**3**)[b]	δ_C(**4**)[a]	δ_C(**5**)[a]
1	34.2 t	34.2 t	125.0 d	101.2 d	130.0 d
2	76.7 d	77.0 d	158.1 s	155.2 s	128.7 d
3	106.7 s	104.0 s	200.1 s	83.1 s	76.0 d
4	53.7 d	55.1 d	81.9 s	86.1 s	31.6 t
5	67.4 s	65.5 s	71.0 s	76.8 s	87.0 s
6	39.2 t	38.6 t	34.0 t	35.8 t	43.3 d
7	20.6 t	20.1 t	20.0 t	20.1 t	27.1 t
8	50.2 t	50.1 t	54.1 t	54.2 t	67.6 t
10	55.1 t	54.9 t	47.8 t	48.1 t	63.3 t
11	76.1 d	76.2 d	32.4 t	31.0 t	23.0 t
12	132.4 s	132.4 s	130.7 s	131.8 s	35.6 t
13	128.6 s	128.5 s	134.3 s	133.4 s	135.0 s
14	110.1 d	110.0 d	108.2 d	108.0 d	128.2 s
15	147.4 s	146.8 s	146.7 s	144.9 s	111.1d
16	146.8 s	147.3 s	147.4 s	145.6 s	146.9 s
17	106.7 d	106.0 d	110.1 d	109.2 d	148.0 s
18					112.7 d
OCH_2O	101.5 t	101.5 t	101.8 t	100.6 t	102.4 t
2-OCH_3	–	–	57.5 q	56.9 q	
3-OCH_3					56.1 q
CH_3CO	–	–	–	168.9 s	
CH_3CO				20.2 q	

Alkaloids **1**, **2**, **4** and **5** recorded in acetone-d_6; **3** in DMSO-d_6

[a] Recorded at 100 MHz

[b] Recorded at 150 MHz

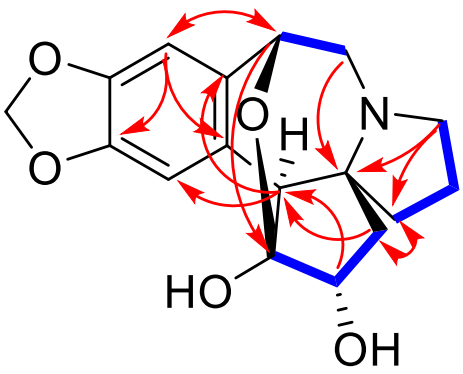

Fig. 2 Key ^1H–^1H COSY (—) and HMBC (⌒) correlations of compound **1**. (Color figure online)

and identified by Dr. Jie Cai, respectively. Two voucher specimen (cai20131002 and cai20140501) was preserved in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

3.3 Extraction and Isolation of *C. lanceolata* and *C. fortunei var. alpina*

The air-dried and powdered leaves and stems of *C. lanceolata* (19 kg) and *C. fortunei var. alpina* (39 kg) was extracted with MeOH (3 × 50 L, 3 × 100 L, 2 days each) at room temperature, respectively, and the solvent was evaporated in vacuo. The extract was dissolved in 1 % HCl solution (v/v) to pH 2–3, basified with 10 % ammonia solution (v/v) to pH 7–8, and partitioned with EtOAc to afford the crude alkaloids (39 and 198 g).

The alkaloidal extract of *C. lanceolata* (39 g) was subjected to CC over silica gel (400 g) and eluted with a $CHCl_3$–MeOH gradient (1:0 to 0:1, v/v) to give four fractions (I-IV) based on TLC analysis. Fraction I (7.5 g) was subjected to C_{18} MPLC with MeOH–H_2O (20:80 to 100:0, V/V) as the eluent to obtain four fractions (I-1–I-4). I-1 (800 mg) was further separated on a C_{18} MPLC with a gradient of MeOH–H_2O (20:80 to 40:60, v/v) and then separated on a preparative C_{18} column with a gradient MeOH–H_2O (30:70 to 40:60, v/v) to afford **6** (30 mg). I-2 (3 g) was purified on a C_{18} MPLC with a gradient of MeOH–H_2O (30:20 to 60:40, v/v) to afford the alkaloid **7** (8 mg). **11** (33 mg) was crystallized from I-3 (1 g), and the mother liquid of this fraction was separated on a C_{18} MPLC with a gradient of MeOH–H_2O (40:60 to 70:30, v/v) to afford the alkaloids **16** (18 mg) and **18** (14 mg). I-4 (2 g) was applied to a C_{18} HPLC with a gradient of MeOH–H_2O (50:40 to 80:10, v/v) then separated on a preparative C_{18} column with a gradient MeOH–H_2O (55:45 to 65:35) to obtain **17** (20 mg), **20** (12 mg) and **21** (5.5 mg). Fraction II (15 g) was applied to a C_{18} MPLC with a gradient of MeOH–H_2O (20:80–100:0, v/v) to obtain four subfractions II-1–II-4. II-1 (5 g) was further applied to a C_{18} MPLC with a gradient of MeOH–H_2O (10:90 to 70:30, v/v) to give four fractions II-1-1–II-1-4. II-1-1 (0.8 g) was separated on a C_{18} MPLC with a gradient of MeOH–H_2O (10:90 to 30:70, v/v) and then separated on a preparative C_{18} column with a gradient MeOH–H_2O (25:75 to 35:65, v/v) to give **1** (8 mg) and **2** (12.5 mg). II-1-3 (2 g) was subjected to a C_{18} MPLC with a gradient of MeOH–H_2O (30:70 to 60:40, v/v) and then separated on a preparative C_{18} column with a gradient MeOH–H_2O (48: 52 to 58:42, v/v) to give **12** (55 mg). II-3(4 g) was applied to a C_{18} MPLC with a gradient of MeOH–H_2O (20:80 to 50:50, v/v) to obtain **27** (9.5 mg), and then separated on a preparative C_{18} column with a gradient MeOH–H_2O (38:62 to 48:52, v/v) to give **14** (11 mg). II-4 (3.0 g) was subjected to CC over silica gel (30 g) and eluted with a $CHCl_3$–MeOH gradient (25:1 to 15:1, v/v) and further purified on a preparative C_{18} column with a gradient MeOH–H_2O (50:50 to 60:40, v/v) to give **9** (14 mg). III (12 g) was applied to C_{18} MPLC with a

gradient of MeOH–H_2O (20:80 to 60:40, v/v) to obtain four subfractions III-1-III-4. III-1 (4 g) was separated on a C_{18} MPLC with a gradient of MeOH–H_2O (10:90 to 40:60, v/v) to give **13** (10 mg). III-3 (2.5 g) was separated on a C_{18} MPLC with a gradient of MeOH–H_2O (30:70 to 60:40, v/v) to give **5** (10 mg) and **22** (22 mg).

The alkaloidal extract of *C. fortunei var. alpina* (198 g) was subjected to CC over silica gel (2.0 kg), eluted with $CHCl_3$–MeOH gradient (1:0 to 0:1, v/v) to yield six fractions (I-VI). Fraction II (43 g) was gradually purified C_{18} MPLC with MeOH–H_2O (30:70 to 100:0, V/V), to afford subfractions II-1–II-6. **6** (200 mg) was crystallized from II-1 (7 g), and the mother liquid of this fraction was separated on a C_{18} MPLC with a gradient of MeOH–H_2O (30:70 to 50:50, v/v) to afford **7** (5 mg). II-3 (11 g) was subjected to CC over silica gel (120 g) with $CHCl_3$–Me_2CO(20:1 to 5:1, v/v) as the eluent and then further purified on a C_{18} MPLC with a gradient of MeOH–H_2O (30:70 to 50:50, v/v) to afford **3** (5 mg). II-4 (8 g) was gradually purified on a C_{18} MPLC (MeOH–H_2O, 40:60 to 60:40, v/v) to afford **20** (98 mg) and then further purified on a preparative C_{18} column with a gradient MeOH–H_2O (48: 52 to 58:42, v/v) to give **21** (17 mg). II-5 (7 g) was separated by C_{18} MPLC with a gradient of MeOH–H_2O (50:50 to 70:30, v/v) to give **23** (39 mg). Fraction III (41 g) was separated on a C_{18} MPLC with a gradient of MeOH–H_2O (20:80 to 100:0, v/v) to afford subfractions (III-1–III-5). Subfraction III-3 (12 g) was gradually separated on a C_{18} MPLC, eluted with MeOH–H_2O (30:70 to 50:50, v/v) to afford **14** (141 mg). **12** (133 mg) was crystallized from III-5 (13 g), and the mother liquid of this fraction was separated on a C_{18} MPLC with a gradient of MeOH–H_2O (20:80 to 40:60, v/v) to afford **10** (32 mg). IV (31 g) was separated on a C_{18} MPLC with a gradient of MeOH–H_2O (10:90 to 100:0, v/v) to yield subfractions IV-1–IV-9. IV-2(8 g) was further purified on a C_{18} MPLC with CH_3CN–H_2O (5:95 to 15:85, v/v) as the eluent to give **7** (200 mg). IV-3 (3 g) was subjected to a C_{18} MPLC with MeOH–H_2O (20:80 to 50:50, v/v), then further purified on a preparative C_{18} column with a gradient MeOH–H_2O (35:65 to 45:55, v/v) to give **26** (46) and **27** (18 mg). **25** (54 mg) was crystallized from IV-5 (13 g). IV-9 (5 g) was gradually separated on a C_{18} MPLC, eluted with MeOH–H_2O (35:65 to 55:45, v/v) to afford **28** (100 mg) and **29** (380 mg). V (25 g) was subjected to a C_{18} MPLC with a gradient of MeOH–H_2O (10:90 to 100:0, v/v) to give five subfractions (V-1–V-5). V-2 (4 g) was separated on a C_{18} MPLC with a gradient of MeOH–H_2O (10:90 to 30:70, v/v) to afford **4** (600 mg). VI (17 g) was purified on C_{18} MPLC with a gradient of MeOH–H_2O (10:90 to 100:0, v/v), and VI-3 (3 g) was gradually purified on a C_{18} MPLC (MeOH–H_2O, 10:90 to 30:70, v/v) and further purified on a preparative C_{18} column with a gradient MeOH–H_2O (15: 85 to 25:75, v/v) to yield **15** (4 mg), **24** (5 mg) and **19** (18 mg).

Cephalotine A (**1**): white powder; $[\alpha]_D^{25}$-31.5 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.01), 291 (3.91) nm; IR (KBr) ν_{max} 3520, 3406, 1631, 1500, 1482, 1342 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (100 MHz) data (acetone-d_6), see Tables 1 and 2; positive HRESIMS *m/z* 318.1336 (calcd for $C_{17}H_{20}NO_5$ $[M+H]^+$, 318.1342).

Cephalotine B (**2**): white powder; $[\alpha]_D^{25}$ −35.8 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 204 (2.95), 291 (3.81) nm; IR (KBr) ν_{max} 3450, 3430, 1631, 1484, 1342 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (100 MHz) data (acetone-d_6), see Tables 1 and 2; positive HRESIMS *m/z* 318.1335 (calcd for $C_{17}H_{20}NO_5$ $[M+H]^+$, 318.1342).

Cephalotine C (**3**): brown oil; $[\alpha]_D^{25}$ +9.0 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ε) 237(3.67), 280 (3.80) nm; IR (KBr) ν_{max} 3437, 2954, 1752, 1735, 1654, 1223 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) data (DMSO-d_6), see Tables 1 and 2; positive HRESIMS *m/z* 330.1337 (calcd for $C_{18}H_{20}NO_5$ $[M+H]^+$, 330.1336).

Cephalotine D (**4**): colorless powder; $[\alpha]_D^{25}$ +138.0 (*c* 0.41, MeOH); UV (MeOH) λ_{max} (log ε) 240 (3.84), 279 (3.89) nm; IR (KBr) ν_{max} 3437, 2922, 1659, 1590, 1130 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (100 MHz) data (acetone-d_6), see Tables 1 and 2; positive HRESIMS *m/z* 374.1604 (calcd for $C_{20}H_{24}NO_6$ $[M+H]^+$, 374.1598).

Cephalotine E (**5**): white powder; $[\alpha]_D^{25}$ +46.3 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.66), 243 (2.73), 288 (2.67)nm; IR (KBr) ν_{max} 3419, 2934, 1623, 1507, 1490 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (100 MHz) data (acetone-d_6), see Tables 1 and 2; positive HRESIMS *m/z* 330.1717 (calcd for $C_{20}H_{24}NO_4$ $[M+H]^+$, 330.1705).

3.4 Cytotoxicity Assay

Three human cancer cell lines, HeLa, SGC-7901 gastric cancer, and A-549 lung cancer, were used in the cytotoxicity assay. All the cells were cultured in RPMI-1640 or DMEM media (Hyclone, USA), supplemented with 10 % fetal bovine serum (Hyclone, USA) in 5 % CO_2 at 37 °C. The cytotoxicity assay was performed according to the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) method in 96-well microplates. Briefly, 100 μL adherent cells were seeded into each well of 96-well cell culture plates and allowed to adhere for 12 h before addition of the test compound/drug. Meanwhile suspended cells were seeded with initial density of 1×10^5 cells/mL just before addition of the test compound/drug. Each tumor cell line was exposed to the test compound at concentrations of 0.06, 0.32, 1.60, 8.0, and 40 μM for 48 h. Each of these tests was conducted in triplicate, with cisplatin (sigma, USA) as the positive control. After the end of the treatment period, cell viability was measured and cell growth curve was plotted.

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Compliance with Ethical Standards

Conflict of Interest The authors declare no conflict of interest.

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